Isolation of a New C_{14} Hydrocarbon from North Indian Vetiver Oil

Recent communications[1,2] report on the isolation of many new terpenoids from vetiver oil (*Vetiveria*, Zizanioides Linn, Bharatpur variety). Punjab variety of the oil has been also studied[3] and its over-all resemblance to the Bharatpur variety has been established. The present paper reports on the isolation and partial characterization of a new C_{14} hydrocarbon from the Punjab variety of the vetiver oil. The leavo-rotatory hydrocarbon fraction of the oil $[\alpha]_D^{22} - 30°$ was isolated by column chromatography of the oil over alumina. To learn about the nature of the carbon skeletons, the hydrocarbon mixture was dehydrogenated with selenium. On the basis of TLC on plates of silica gel G impregnated with trinitrobenzene, the dehydrogenation mixture was shown to consist of mainly Cadalene (I) and 1,6-dimethyl-4-ethyl naphthalene (II) in the ratio (70:30% VPC) respectively. 1,6-Dimethyl-4-ethyl naphthalene points to the possibility of the presence of C_{14} hydrocarbons with the rare khusilane[4,5] (III) carbon skeleton. Isolation of the hitherto unknown C_{14} diethynoid dextro-rotatory hydrocarbon in a pure form was possible by elaborate column chromatography over silica gel impregnated with silver nitrate. The course of separation was carefully followed by subjecting each fraction to dehydrogenation. The fractions which gave mainly 1,6-dimethyl-4-ethyl naphthalene were mixed together. This gave a dextro-rotatory fraction which showed the presence of at least 3 clearly visible spots, one being major. The major component was isolated by preparative TLC to afford a new hydrocarbon $C_{14}H_{22}$ (found: C, 88.15; H, 11.98. $C_{14}H_{22}$ requires C, 88.35; H, 11.65%), bp 115° bath/2 mm, $[\alpha]_D^{20} +124°$. Its IR-spectrum showed the presence of both methylenic (>C=CH_2, 3075, 1642 and 892 cm^{-1}) and trisubstituted double bond (>C=C<H, 1665 and 835 cm^{-1}). The hydrocarbon on dehydrogenation afforded 1,6-dimethyl-4-ethyl nephthalene (II) as confirmed by mp and mixed mp determination of the TNB complex with an authentic sample. Presence of 2 double bonds was confirmed by catalytic hydrogenation which afforded a tetrahydroproduct $C_{14}H_{26}$. The IR-spectrum of the tetrahydroderivative was superimposable on that of khusilane[4] (III) earlier prepared from the aldehyde khusilal (IV). These data, therefore, conclusively show that the new hydrocarbon is another rare nor-terpenoid with the khusilane carbon framework. This is the first report of the isolation of a C_{14} hydrocarbon. Work on structure determination of this hydrocarbon is in progress and will be published separately.

Zusammenfassung. Isolierung und Strukturaufklärung eines ungewöhnlichen Terpen-Kohlenwasserstoffs aus nordindischem Vetiver-Öl.

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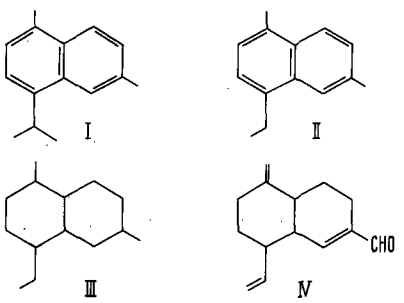

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Effect of Ammonium Ions on the Growth of *Aspergillus nidulans*[1]

In earlier studies[2] we have shown that one of the indirect effects of biotin deficiency is an increase in protein content of the mould *Aspergillus nidulans*. In continuation of these studies we observed that there is more utilization of ammonium ions in biotin-deficient culture as compared with normal, thus indicating a relation between the biotin status of the culture and the cellular permeability.

Earlier KINOSHITA[3], while studying glutamic acid fermentation in *Micrococcus glutamicus*, has reported that in biotin-deficient cells glutamate can rapidly flow out of the cell, due to the change in cell permeability. Later GAVIN and UMBREIT[4] demonstrated that biotin deficiency in *Escherichia coli* caused a change of the permeability barrier evidenced by leakage of internal solutes, such as glutamic acid, and penetration of impermeable substances. Therefore in the present investigation we have made a comparative study in the normal and biotin-deficient *A. nidulans* of the following: a) relative growth pattern of the mould on different nitrogen sources, b) ammonium ion utilization, and c) lipid content of the cell wall.

The media composition, cultural conditions and harvesting of the mould was similar to that described earlier[2]. Ammonia was determined by the method of FAWCETT and SCOTT[5]. Nitrate reductase activity was determined

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Table I. Ammonium ion utilization and its effect on the growth of normal and biotin-deficient *Aspergillus nidulans*

Biotin status	Ammonium[a] utilization (%)	Growth on different nitrogen sources, mycelial dry weight (mg)				
		NH_4NO_3	$(NH_4)_2SO_4$	KNO_3	$NaNO_3$	NH_4NO_3[b] and KNO_3
Normal	48.0	420	270	840	859	500
Deficient	60.0	740	500	854	862	760

[a] Substrate is NH_4NO_3. [b] Substituted in terms of nitrogen content.

Table II. Glutamic pyruvic transaminase (GPT), nitrate reductase activity and cell wall lipids of normal and biotin-deficient *Aspergillus nidulans*

Biotin status	GPT activity[a] (units/mg protein)	Nitrate reductase (units/mg protein)				Cell wall[c] lipids (%)
		KNO_3	NH_4NO_3	$(NH_4)_2SO_4$	NH_4NO_3[b] and KNO_3	
Normal	23.0	40.0	20.0	0.0	24.0	5.0
Deficient	45.0	38.0	18.0	0.0	20.0	3.0

[a] A unit of enzyme is that amount of the enzyme which produces 1 µg of alanine in 1 h at 30 °C. [b] Substituted in terms of nitrogen content. [c] Substrate is NH_4NO_3.

colorimetrically as described by COVE[6]. Protein was measured by the method of LOWRY et al.[7]. Preparation and purification of cell walls were made by following the method of SHAH and KNIGHT[8]. Lipids from the cell-wall preparations were extracted once with cold ethanol, twice with ethanol-ether (1:3 v/v) mixture at room temperature, and then with ethyl-ether at room temperature. The combined solvent fractions were concentrated, the residual material extracted twice with ethyl-ether and the ether extract dried and weighed. That the mould was biotin-deficient was confirmed by microbiological assay of the mould extract by the method of SKEGGS[9] using *Lactobacillus arabinosus* as the test organism.

Results listed in Table I indicate that in biotin-deficient culture ammonium utilization is increased by 25%, which might be one of the causes of more cellular protein synthesis and hence the growth. Further studies have been carried out with different nitrogen sources, such as KNO_3, $NaNO_3$, $(NH_4)_2SO_4$ and NH_4Cl. When K or Na cation was used there was no difference in the mat weights of the normal and biotin-deficient cultures. But when partially or completely substituted by ammonium ion in terms of their nitrogen content, then there was a difference in normal and biotin-deficient mat weights; in the absence of nitrate ions as expected, the growth is further reduced.

Biotin deficiency in *A. nidulans* resulted in significant increase in glutamic-pyruvic transaminase activity (Table II). Similar increase was observed in the case of glutamic oxaloacetate transaminase[2] also. It is possible that an increase in transaminases and glutamic dehydrogenase[10] activities may be responsible for overall increase in protein synthesis in biotin-deficient mould. It is known that ammonia represses nitrate reducase activity[6]. The results recorded in Table II also confirm that in the presence of ammonia, nitrate reductase activity is repressed, thus inhibiting the conversion of nitrate to ammonia. In the absence of ammonium ions, high nitrate reductase activity was detected. The normal culture grown on medium containing NH_4NO_3 shows less growth, due to less utilization of ammonium ions as well as the reduction in transaminase, glutamic dehydrogenase and nitrate reductase activities. In biotin-deficient culture, the availability of ammonia is greater, may be due to the change in the cell permeability, perhaps because of the less lipid synthesis. In view of this, cell-wall lipid of the normal and biotin-deficient cultures were determined. It was found that in the cell walls of biotin-deficient culture there is approximately 40% reduction in lipid content. It seems that, due to this, the molecular structure of the membrane, particularly that of the lipid layer, might be so affected as to facilitate the entry of ammonium ions, eventually leading to more ammonia utilization.

Zusammenfassung. In einer Biotin-Mangelmutante von *Aspergillus nidulans* wurde, verglichen mit dem der Normalkultur, ein grösserer Ammonium-Verbrauch gefunden. Dies scheint seine Ursache in der Veränderung der Permeabilität der Zellwand zu haben, deren Lipoidgehalt um 40% niedriger ist. Eine Zunahme der Transaminasen (GOT, GPT) und Glutamindehydrogenase könnte für eine höhere Proteinsynthese in Mangelstamm verantwortlich sein.

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